Identification of tolerance to *Fusarium solani* in *Pisum sativum* ssp. *elatius*

Hance, S.T., Grey, W. and Weeden, N.F

Dept. of Plant Sci. and Plant Path.
Montana State Univ., Bozeman, MT, USA

Fusarium root rot, produced by *Fusarium solani* f. sp. *pisi* is an important disease of pea in many regions of the world (4, 6). Complete resistance to this disease has not been reported in pea, but a number of sources of partial tolerance have been found (4). Recently, Grünwald et al. (2) screened the Pisum Core Collection and identified 44 accessions with some level of tolerance (mean disease severity rating of 2.5 or less on a scale of 0 to 5). Most of these accessions were purple-flowered *P. sativum* ssp. *sativum* lines, a number of which originated from Afghanistan. None of the tolerant lines were identified as being *P. s.* ssp. *elatius* or *P. s.* ssp. *abyssinicum*. We were therefore surprised to find that in a small screen of lines we have used as parents in mapping populations, a *P. s.* ssp. *elatius* line (JI 1794) exhibited good tolerance to this pathogen. Here we present the results of an analysis designed to locate the primary genetic factors responsible for the increased tolerance in JI 1794.

Materials and Methods

The population used for this study was the recombinant inbred line (RIL) population derived from a cross between JI 1794 (*Pisum sativum* ssp. *elatius* var. *pumilo*) and Slow, (*Pisum sativum* ssp. *sativum*). This population consists of 51 F_2-derived F_{10+} lines with excellent marker coverage for genetic mapping. It has been used to form the basis of the consensus map of pea (11). A standard susceptible control line (Dark Skin Perfection) and an accession from Afghanistan known to possess a good level of tolerance (PI 223285) were also used in the analysis.

The cultures of *Fusarium solani* used in this experiment are referred to as strain 915 and were isolated from the pea trial fields on Spillman Farm in Pullman, WA with the assistance of K. McPhee and D. Mathre. The soil used for the inoculum was a 3:1 river sand and silt loam mixture that had been sterilized. Inoculum soil was prepared according to a method previously described (5), except that Czapek-Dox broth was used in place of Kerr's liquid medium, and the inoculated soil was mixed by hand.

Pasteurized soil mix, consisting of equal parts silt loam, river sand, and Sunshine peat moss, was obtained from the Plant Growth Center at Montana State University. The soil was placed in ten flats to a depth of 5 cm. Five seeds for each pea line (a susceptible control [Dark Green Perfection], a tolerant control from Afghanistan [PI223285], the two parents and 51 RILs) were collected and surface sterilized for five minutes in 10% chlorine bleach. The seed coats were then nicked and placed between moistened filter paper and allowed to imbibe for 12 hrs. The seeds were placed on the soil surface in the flats (in a predetermined randomized pattern), and 2 grams of inoculum were placed on top of each seed. An additional 2 cm layer of soil mix was added over the inoculated seeds, and the flats were thoroughly watered. Flats were watered to bring soil to field capacity. Soil moisture was maintained between field capacity and leaf wilting point in order to avoid over saturation and the development of damping off diseases such as Pythium. Greenhouse conditions provided 14 hours of light per day, four of which (two in the morning and two in the afternoon) were supplemented by growth lights.

Disease incidence was determined using two methods: a disease score and a tolerance score.

***Disease score*:** Plants were carefully extracted from the soil after four weeks, and the roots were thoroughly rinsed. Symptoms of discoloration and disfiguration of the roots and epicotyl were scored visually on a scale of 0 to 5 (3), with 0 indicating negligible evidence of disease and 5 indicating complete rot, as shown in Fig. 1. Within most lines the disease score was relatively consistent ($\pm$1 unit on our scale). An average score for each line was calculated and used for analysis and genetic mapping.

***Tolerance score*:** During the analysis a number of plants were observed that despite a high disease score displayed vigorous vegetative growth, and we were uncertain whether we were capturing all the disease effects by simply using the traditional disease score. We therefore developed a second scoring method we designate the 'tolerance score' in which the vigor of the vegetative growth is also taken in to account. The tolerance score differed from the disease score in that the original score given to each plant was increased, decreased, or left the same depending on the correlation (or lack thereof) of the above ground symptoms to the expected above ground symptoms based on the disease score. For example, for a plant with a disease score of 4, the above ground portion would be expected to be wilted or drying out. If that were the observed case, the score of 4 would be retained for the tolerance score. If the above ground portion of the plant appeared healthy (a condition expected for a disease score of 2), then the tolerance score would be changed to a 3, thereby averaging the scores for observed and expected symptoms.

Fig. 1. Scores from 0 to 5, from left to right

The disease incidence data were analyzed in two ways. The ten most tolerant lines and the ten most susceptible lines were used as the tails of the distribution and were analyzed for correlation with segregating marker loci using QUIKMAP (10). For each locus that displayed significant skewing (7 or more tolerant lines with the allele from one parent and 7 or more susceptible lines possessing the allele from the other parent) the entire population was divided into two groups based on genotype at that locus. An average tolerance score for each group was calculated and the difference tested for significance using a two-tailed Student's t-test. The second approach was a traditional analysis for quantitative trait loci using QTL Cartographer (9) and a set of nearly 200 loci spaced about 4 cM apart along each of the linkage groups.

Results

Most lines germinated well, and the plants appeared healthy above ground throughout the experiment. However, four lines displayed poor germination. Two of these lines lacked overall vigor even when growing in clean soil, and the poor germination could be attributed to poor seed quality. However, the other two lines gave excellent germination and emergence when placed in pasteurized soil. Because root symptoms could not be adequately assessed for these lines, we did not include the two 'low vigor' lines in our final analyses and performed the two QTL analyses both with and without data from the other two lines (the few plants from these lines that did emerge displayed a highly susceptible phenotype of 4 or 5).

For each plant, disease symptoms were scored as described above. Average disease scores ranged from 1.10 to 4.85, with JI 1794 scoring 1.25 and Slow scoring 3.30. The susceptible control gave an average disease score of 3.90 and PI223285 gave an average score (1.35) very similar to JI 1794. Average tolerance

scores ranged from 1.10 to 4.85, with JI 1794 scoring 1.06 and Slow scoring 2.85. Scores for individual RILs are listed in Table 1.

The QUIKMAP analysis revealed four potential regions on the linkage map correlating with the fluctuation in susceptibility. These regions included the portion of LG III around *M*, a portion of LG III near *Le*, a section of LG IV just proximal to the ribosomal array and the region on LG VI distal to *Gty*. Only the last of these regions showed a significant difference between the averages for the two alleles using Student's t-test (average disease score for lines with JI 1794 allele = 2.75 and for lines with the Slow allele = 3.29; P = 0.02). Values for the average tolerance scores were similar (JI 1794 = 2.69, Slow allele = 3.20; P = 0.03). The region on LG IV showed a P = 0.08 for both the average disease score and the average tolerance score, while the other two regions had P > 0.10 for both averages. The two-locus average genotypic scores (j = JI 1794 allele and s = Slow allele with the locus on LG VI given first) are as follows jj = 2.44, js = 3.08, sj = 3.29 and ss = 3.29, indicating that the locus on LG VI had the major effect, with the j allele on LG IV providing additional tolerance in the presence of the j allele on LG VI.

The analysis using QTL Cartographer identified three QTLs with an LR of 11.5 (= LOD 2.5) or greater (Fig 2). These three QTLs corresponded to the regions on LG VI and LG IV revealed in the QUIKMAP analysis, as well as that near *Le* on LG III. In contrast to the QUIKMAP analysis, QTL Cartographer indicated a greater effect by the region on LG IV.

Table 1. Average and weighted average scores of pea lines

Pea Line	Average Disease Score	Average Tolerance Score
87-18I-a	3.40	3.30
87-18I-c	3.10	3.10
87-18I-d	2.20	2.10
87-18I-j	2.00	2.00
87-18I-l*	4.85	4.85
87-18I-m	3.30	3.40
87-18I-n	3.00	3.00
87-18I-o	2.67	2.42
87-18I-p	2.13	2.90
87-18I-q	3.35	3.25
87-18I-s	4.00	3.85
87-18I-u	3.10	2.90
87-18I-v	3.50	3.25
87-18I-w	4.10	4.05
87-18I-x	2.30	2.20
87-18i-d	3.70	3.45
87-18i-e	3.30	3.40
87-18i-f	2.80	2.55
87-18i-g	2.10	1.90
87-18i-h	2.00	1.80
87-19I-a	3.00	3.05
87-19I-b	3.40	3.05
87-19I-c	1.50	1.30
87-19I-d	3.70	3.45
87-19I-e	3.40	3.50
87-19I-f	2.50	2.30
87-19I-g	2.80	2.70
87-19I-h	3.40	3.65
87-19I-i	2.20	2.10
87-19I-j	2.80	2.45
87-19I-k	2.10	2.25
87-19I-l	3.80	3.90
87-19I-m	2.70	2.50
87-19I-n*	4.50	4.50
87-19I-o	3.67	3.75
87-19I-p	2.60	2.55
87-19I-s	2.40	2.55
87-19I-t	3.00	3.15
87-19I-u	4.50	4.33
87-19I-v	4.00	4.00
87-19i-a	2.90	2.55
87-19i-b	2.30	2.20
87-19i-c	2.90	2.65
87-19i-e	3.70	3.30
87-19i-f	1.10	1.10
87-19i-g	3.70	3.35
87-19i-h	3.50	3.45
87-19i-i	3.50	3.55
87-19i-j	3.10	2.65

*Line germinated poorly in experiment

Discussion

The susceptibility segregation patterns observed in this study, and the analyses thereof, indicate that tolerance to *F. solani* is multigenic in JI 1794. Both methods of assessing susceptibility (disease score and tolerance score) identified the same regions of the genome as influencing the trait.

Because the former method is traditional and less subjective, we recommend the continued use of the disease score as the standard analysis. The QUIKMAP analysis and QTL Cartographer also gave very similar results, although QUIKMAP revealed only one region with a significant effect. The effects of the regions on LG IV and LG VI are particularly strong, and we are currently introgressing these regions into a more acceptable genetic background for commercial use.

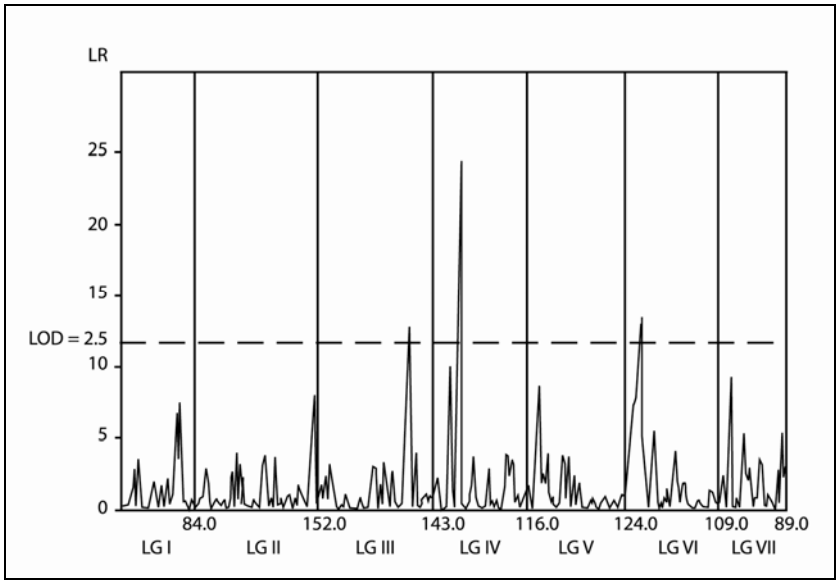

Fig. 2. Chromosome scan generated by QTL Cartographer (9) displaying the position of those regions of the JI1794 x Slow linkage map with significant effect on tolerance to Fusarium root rot. Linkage groups are labeled at the bottom of the figure and separated by vertical lines. The scale on the vertical axis is the "likelihood ratio" (LR) The horizontal dashed line indicates the LR of 11.5, corresponding to an LOD of 2.5, which was taken as the threshold for significance.

The position on the linkage map of the factors influencing tolerance in this study may provide some indication as to what these genes might be. There are several genes influencing susceptibility to fungal diseases on LG VI (1, 7, 8). However, at least *Er1* and the QTL identified for tolerance to Aphanomyces root rot are located on the opposite side of *Gty* from the QTL for Fusarium root rot. Indeed, the position of *Er1* has been accurately determined in the same JI 1794 x Slow RIL population used in this study, and several recombinant lines can be identified. Similarly, the position of the Fusarium root rot QTL on LG IV is nearly 50 cM from the Aphanomyces root rot QTL reported on this same chromosome (12). JI 1794 is known to have smaller roots than Slow, and a major gene influencing this trait is located very near *Le* (13). The smaller root mass of JI 1794 could, in some way, be responsible for the QTL for Fusarium root rot tolerance identified in this study to be near *Le*. Other than this possible connection between *Le* and the minor QTL, there do not appear to be any obvious candidate genes that could explain the tolerance found in JI 1794.

The primary source of Fusarium root rot tolerance reported in pea has been accessions from Afghanistan (2). The genetic factors responsible for this tolerance have not been analyzed extensively, and we do not know if the genes found in JI 1794 are different from those present in the Afghanistan material. An analysis of allozyme diversity in pea (14) indicated that the Afghanistan material formed a unique subset of the *Pisum sativum* ssp. *sativum* germplasm, not closely related to *P. s.* ssp. *elatius*. Hence, Fusarium root rot tolerance either arose independently in the two lineages or dates back to a very early *Pisum sativum* form and has subsequently been lost in most other pea lines.

1. Cargnoni, T.L., Weeden, N.F. and Gritton, E.T. 1994. Pisum Genetics 26: 11-12.
2. Grünwald, N.J., Coffman, V.A. and Kraft, J.M. 2003. Plant Dis. 87: 1197-1200.
3. Kraft, J.M. 1989. Pisum Newslett. 21: 82-85.
4. Kraft, J.M., Haware, M.P., and Hussein, M.M. 1988. In: Summerfield, R.J. (ed.) World Crops: Cool Season Food Legumes. Kluwer Academic Press, Boston. pp 565-575.

5. Kraft, J.M., and Roberts, D.D. 1969. Phytopath. 59: 149-152.
6. Kraft, J.M. and Pfleger, F.L. 2001. Compendium of Pea Diseases and Pests. Second Ed. Amer. Phytopath. Soc., St. Paul, MN, 67 pp.
7. Pilet-Nayel, M.L., Muehlbauer, F.J., McGee, R.J., Kraft, J.M., Baranger, A., and Coyne, C.J. 2002. Theor. Appl. Genet. 106: 28-39.
8. Timmerman, G.M., Frew, T.J., Weeden, N.F., Miller, A.L. and Goulden, D.S. 1994. Theor. Appl. Genet. 88: 1050-1055.
9. Wang, S. and Zeng, Z-B. 2003. Windows QTL Cartographer v2.0. Bioinformatics Research Center. North Carolina State Univ.
10. Weeden, N.F. and Barnard, J. 1994. NYSAES, Geneva, NY. Computer Ctr. Tech. Rpt. 20-2.
11. Weeden, N.F., Ellis, T.H.N., Timmerman-Vaughan, G.M., Święcicki, W.K., Rozov, S.M. and Berdnikov, V.A. 1998. Pisum Genetics 30: 1-4.
12. Weeden, N.F., McGee, R., Grau, C.R. and Muehlbauer, F.J. 2000. Pisum Genetics 32: 53-55.
13. Weeden, N.F. and Moffet, M. 2002. Pisum Genetics 34: 28-31.
14. Weeden, N.F. and Wolko, B. 1988. Measurement of genetic diversity in pea accessions collected near the center of origin of domesticated pea. IPBGR final report, Rome, 20 pp.